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Effect of Aerosolized Anti-IgE (E25) on Airway Responses to Inhaled Allergen in Asthmatic Subjects

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ABSTRACT

TOP
ABSTRACT
ARTICLE
REFERENCES

Intravenous administration of a humanized monoclonal antibody of IgE (E25) attenuates the early and late phase response to inhaled allergen in allergic asthmatic subjects. To test whether direct delivery of E25 to the airway might have the same effect, we conducted a randomized, double-blind, three group study in 33 subjects with mild allergic asthma (20 to 46 yr of age, 21 men, FEV_1 > 70% predicted). The airway responses to aerosolized allergen were determined at baseline, after 2 and 8 wk of once daily treatment with aerosolized placebo (n = 11), aerosolized E25 1 mg (n = 12), or aerosolized E25 10 mg (n = 10), and after 4 wk of treatment withdrawal. We found that E25 was detectable in the serum during aerosol treatment, although serum IgE did not change significantly in any of the three groups during treatment. In addition, both doses of E25 were no more effective than placebo in attenuating the early phase responses to allergen at both times during treatment. Although aerosolized E25 was generally well tolerated, one subject receiving aerosolized E25 10 mg daily was found to have serum IgG and IgA antibodies to E25. We conclude that aerosol administration of an anti-IgE monoclonal antibody does not inhibit the airway responses to inhaled allergen in allergic asthmatic subjects. We speculate that the observed lack of efficacy may be due to the inability of aerosol route of delivery to result in high enough concentrations of E25 in the tissue compartments surrounding IgE effector cells to neutralize IgE arising from local airway and pulmonary sources and IgE arising from the vascular space. Additionally, the aerosol route of delivery of monoclonal antibodies may be more immunogenic than the parenteral route.

ARTICLE

▲ TOP
▲ ABSTRACT
▪ ARTICLE
▼ REFERENCES

Recombinant humanized monoclonal antibody-E25 or "E25" is a nonanaphylactogenic anti-IgE antibody that attenuates both the early and late phase responses to inhaled allergen in asthmatic subjects (1, 2). In this study, we examined whether aerosolized R25 attenuates the airway responses to inhaled allergen in allergic asthmatic subjects. To do this, we conducted a randomized, placebo-controlled, parallel group clinical trial of the effects of 8 wk of once daily treatment with aerosolized E25 in two doses (1 mg and 10 mg) on the early and late phase responses to allergen challenge in allergic subjects with mild asthma.

Thirty-three subjects with asthma with $FEV_1 \geq 70\%$ predicted, bronchial hyperactivity to methacholine, serum IgE < 500 IU/mL, a positive skin prick test to aeroallergens (house dust mite, perennial ryegrass, birch, cat pelt, or horse hair) were studied (Table 1 and Figure 1). Exclusion criteria were the use of any corticosteroids or symptoms of an upper or lower respiratory tract infection in the previous 6 wk, and history of tobacco use (any in the past 12 mo and total use ≥ 10 pack-years). The study protocol and consent form was approved by the committees for human research at each participating institution, and each subject provided written informed consent.

TABLE 1

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CLINICAL CHARACTERISTICS OF THE STUDY SUBJECTS*

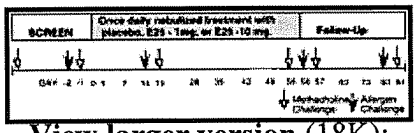

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Figure 1. Diagrammatic representation of the three-phrase, 14- to 16-wk, randomized, double-blind, placebo-controlled, parallel group trial. There was a 2- to 4-wk screening phase, followed by an 8-wk treatment phase, followed by a 4-wk follow-up phase. During the screening phase, subjects were characterized by spirometry, methacholine reactivity, and allergen skin tests, and they were taught to record their peak flows in a diary using a MiniWright Peak Flow Meter (Clement Clarke, Columbus, OH). Subjects then underwent baseline methacholine challenge and airway allergen challenge in the sequence depicted in the diagram, after which they began self-administering study drug (E25-1 mg, E25-10 mg, or matched placebo) once a day by nebulizer for 8 wk. Methacholine challenge and allergen challenge were repeated as shown in the diagram. Spirometry was performed on Days 0, 14, 28, 42, 56, 70, and 84. Samples of venous blood for standard tests of hematologic, renal, and hepatic function were collected once during the screening phase and again on Days 0, 14, 28, 56, and 83. Samples of venous blood for levels of free IgE, total IgE, and rhuMAb-E25 were collected at Days 0, 14, 28, 42, 56, 70, and 83. Finally, bronchoscopy and bronchoalveolar lavage was performed on the 10 subjects enrolled at the Québec center during the screening phase and again on Day 42.

Subjects were randomized to E25-1 mg, E25-10 mg, or matching placebo (E25 excipient [150 mM

NaCl, 10 mM acetate at pH 5.2]); randomization was stratified according to whether the subject had a late phase response to allergen during the screening phase. Subjects self-administered medication once daily at home using a PARI IS-2 nebulizer powered by a PARI Master compressor. On the basis of data from aerosol experiments in monkeys (3), the E25-1 mg dose was predicted to deliver 150 μg to the lower airways and the E25-10 mg dose was predicted to deliver 1.5 mg.

Skin reactivity to house dust mite (*Dermatophagoides pteronynisinus* and *Dermatophagoides farinae*), cat pelt, ryegrass (*Lolium perenne*), birch (*Betula* spp), and histamine (1.8 mg/ml), all from Bayer Pharmaceuticals (Spokane, WA) was assessed, as previously described (1, 2).

Bronchial reactivity to methacholine (4) and allergen (1) was determined as previously described. Allergen challenges during the treatment and follow-up phases were performed similarly to baseline, except that the first allergen concentration was two doubling doses below the allergen concentration causing a 20% fall in FEV_1 at baseline. During the treatment and follow-up phases, the allergen challenge continued until the FEV_1 fell by ≥ 20% or until the same allergen concentration given at baseline was delivered, whichever occurred first.

Total IgE in serum was measured using a microparticle enzyme immunoassay (Abbott Laboratories, Abbott Park, IL). Total IgE in BAL was measured using a more sensitive ELISA (lower limit of detection, 10 pg/ml) as follows: 96-well plates coated overnight with 100 ng of monoclonal anti-IgE antibody in carbonate buffer at pH 9.6 were washed and 100 μl of sample were added. The captured IgE was detected with goat anti-human IgE-biotin (Kirkegaard and Perry, Gaithersburg, MD) and streptavidin-β-galactosidase (Boehringer Mannheim, Indianapolis, IN) followed by 4-methylumbelliferyl-β- D-galactoside substrate (Sigma Chemical Co., St. Louis, MO). The reaction was stopped with 0.3 M glycine at pH 10.5. The fluorescence was read using 360 nm excitation and 460 nm emission wavelength. Free IgE (IgE not in a complex with E25), total E25, free E25, and IgG anti-E25 Fab antibody were measured in blood by ELISA, as previously described (1). Total E25 in serum and BAL samples was measured by ELISA as previously described (1). IgA and IgM class anti-E25 antibodies in blood were assayed similarly using plates coated overnight at 4° C with 300 ng of E25 Fab fragment in 100 μl of PBS. The plates were washed with 0.05% Tween 20 in PBS, then incubated with assay diluent (0.5% BSA/0.05% Tween 20/0.01% thimerosal in PBS) for 1 h to block nonspecific binding sites. Samples were diluted 1/100 in assay diluent, either with added excipient control or with 100 μg/ml E25. The plates were washed and the diluted samples were incubated in the wells for 1 h. The plates were washed and either goat antihuman IgA-HRP conjugate (American Qualex) diluted 1/ 1,000 or goat antihuman IgM-HRP conjugate (Sigma) diluted 1,500 was added. After incubation in the wells for 1 h, the plates were washed and the color was developed using OPD.

Total E25 and IgE concentrations in BAL fluid were corrected for dilution using a urea-based dilution factor. Urea was measured in the BAL using a blood urea nitrogen end point assay kit (Sigma) and a modified protocol for sensitive urea detection (5).

Bronchoscopy was performed as previously described (6). For bronchoalveolar lavage (BAL) three 50-ml boluses of 0.9% saline solution at 37° C were instilled and then aspirated. The BAL fluid was centrifuged at 2 to 8° C for 10 min; 2 ml of the supernatant was aliquoted and diluted with 2 ml of the

BAL diluent (1% BSA/0.1% Tween 20/20 mM phosphate/ 0.9% NaCl). The diluted BAL sample was filtered through a 0.22 μM centrifugal filter (Millipore Ultrafree MC; Millipore Corp., Bedford, MA) at 4,000 × *g* for 5 min.

Data are described as mean and standard deviation or as the geometric mean with 95% confidence intervals, as appropriate. Values for peak flow were analyzed by calculating weekly averages from daily data collections. The area under the curve (AUC) for allergen-induced changes in FEV_1 during the early (0 to 1 h) and late (3 to 7 h) phases was calculated using the trapezoidal rule (percent fall in FEV_1 × minutes). Between- and within-group comparisons were made using Wilcoxon's rank sum and signed-rank tests, respectively. A p value ≤ 0.05, using two-tailed tests, was considered statistically significant.

Thirty-one of the 33 enrolled subjects completed the study. One subject in the placebo arm developed an asthma exacerbation on Day 7 of treatment, and another subject in the placebo arm wihdrew consent on Day 11 because of a change in living location. One additional subject in the placebo group was unable to undergo methacholine on Days 55 and 57 or allergen challenge on Day 56 because of acute labyrinthitis. Overall, aerosolized E25 was well tolerated (Table 2). There were no serious adverse events during the study, and there were no statistically significant differences among treatment groups in the incidence of adverse events. However, whereas only three of 11 subjects in the placebo group reported headache, nine of 12 subjects in the 1 mg group and eight of 10 subjects in the 10 mg group reported headache during the treatment phase.

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TABLE 2

PULMONARY FUNCTION AT BASELINE, AT THE END OF TREATMENT, AND DURING FOLLOW-UP*

Compliance was assessed by counting used and unused medication vials at each study visit during the treatment phase. Using this measure of compliance, we found that nine of the 12 subjects in the placebo group, 11 of the 12 subjects in the 1 mg group, and nine of the 10 subjects in the 10 mg group completed at least 90% of all 56 treatments.

Serum total IgE levels (free IgE + IgE complexed to E25) did not change significantly during the treatment phase, and serum-free IgE concentrations were similarly unaffected (data not shown). Changes in concentrations of serum total or free IgE were not expected because of the low serum concentrations of E25. Concentrations of serum E25 were insufficient to complex significant amounts of serum IgE.

The concentration of allergen delivered during the treatment period was similar to the concentration delivered at baseline in all the treatment groups. For example, in the E25-10 mg group, the concentration of allergen delivered on Day 56 was 0.10 doubling doses less on average (standard deviation of 0.32 doubling doses) than at baseline; the corresponding doubling dose values for the E25-1 mg group and the placebo were 0.58 (2.07) and 0.25 (0.71), respectively; p = 0.78 between groups. Treatment with E25-1 mg was associated with a significant within-group attenuation in the

early phase response to allergen, but this change was not significantly greater than placebo, and the E25-10 mg treatment group did not show any significant within- or between-group effect (Figure 2 and Table 3). Two subjects in the placebo group, four subjects in the E25-1 mg group, and four subjects in the E25-10 mg group had a late phase response to allergen. In this small subgroup of subjects, there was no statistically significant difference in the magnitude of the late phase response during treatment in any group (Figure 3, Table 3).

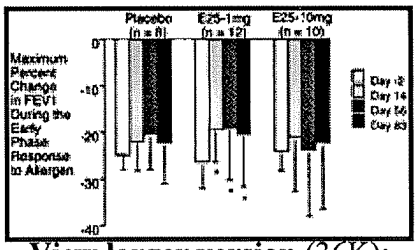

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Figure 2. Effect of aerosolized E25 on the change in FEV_1 observed during the first hour after allergen challenge (early phase response) in the three treatment groups for each of the four allergen challenges performed during the study (*see* Figure 1). Data are presented as mean ± SD. *Asterisks* denote significantly different from Day −2, but not significantly different from the change seen in the placebo group).

TABLE 3

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EFFECTS OF E25 AND PLACEBO ON EARLY AND LATE PHASE AIRWAY RESPONSES ASSESSED AS AREA UNDER THE CURVE FOR CHANGE IN FEV_1 DURING ALLERGEN CHALLENGE*

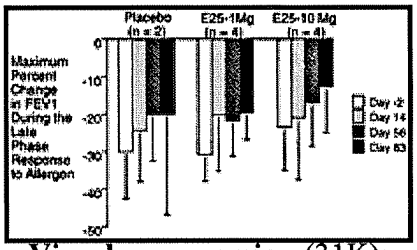

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Figure 3. Effect of aerosolized E25 on the change in FEV_1 observed between 3 and 7 h after allergen challenge (late asthmatic response) in the subgroup of subjects in each treatment group who demonstrated a significant late phase response during the screening phase. Data are presented as mean ± SD.

E25 was undetectable in serum samples from the placebo group except for a single observation on Day 0 (Table 4). Serum levels of E25 were detectable in both the low dose (1 mg) and the high dose (10 mg) groups. In the low dose group, detectable serum levels of E25 were found in four of the 12 subjects at some point during the study period (Table 4). In the high dose group, a larger production of subjects had detectable serum E25 levels, especially during the initial treatment period when all 10 of the subjects had detectable levels (Table 4 and Figure 4). Notably, the frequency with which E25 levels were detectable in the E25-10 mg group declined during the treatment phase from 10/10 to 6/10 (Table 4 and Figure 4). The number of empty medication vials returned by subjects in the high dose group was similar for all treatment visits.

TABLE 4

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NUMBER OF SUBJECTS WITH DETECTABLE SERUM E25 CONCENT

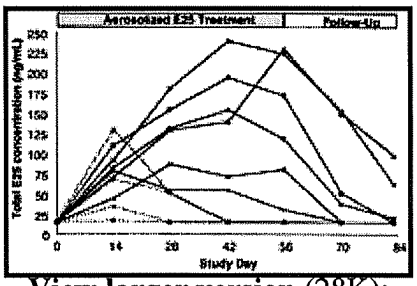

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Figure 4. E25 levels in blood from the 10 subjects in the 10 mg group at different time points during the 8-wk treatment period and during the 4-wk follow-up period. Four subjects (*dashed lines*) had E25 levels that were undetectable by Day 42 of treatment despite higher levels earlier in the treatment period.

A serum IgG antibody directed against E25 was detected at Day 28 of treatment in one subject (Subject 0204) in the high dose E25 group (Table 5); antibodies were not detected in any other subject in any other group. This finding coincided with a decline in serum E25 concentration in this subject. The antibody remained detectable at the end of the follow-up period (Day 83), but it was undetectable during an additional special follow-up visit 11 wk after study completion. Clinical examination, chest radiograph, pulmonary function tests (including a test of diffusing capacity), and analysis of blood and urine did not reveal any evidence of immune-complex-mediated disease in this subject 11 wk after study completion.

TABLE 5

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LEVELS OF E25 AND IgG ANTI-E25 IN SUBJECT 0204 IN THE E25-10 mg GROUP

Four subjects in the 10 mg group had no detectable E25 in blood at Day 56 (Figure 4). Blood samples from these four subjects were extensively studied for anti-E25 antibodies of IgG, IgA, and IgM classes. The subject who had an IgG anti-E25 response also had an IgA anti-E25 response. No significant reactivity of any antibody class was found in the other three subjects.

The levels of E25 in BAL were highly variable (Table 6) but generally within the range expected from data available from a preclinical aerosol study in cynomolgus monkeys (Theresa Sweeney, Ph.D, personal communication). Notably, one of the subjects in the 10-mg group had no detectable E25 in BAL on Day 42. Serum levels of E25 were also undetectable for this subject from Day 28 onward. No positive anti-E25, IgG, IgA, or IgM antibody titers were detected in this subject, and there was no indication of noncompliance. The disappearance of detectable drug levels in the lung and serum may still be due to noncompliance or to anti-E25 antibodies of IgG, IgA, or IgM isotypes not identified by the immunoassays used here. Total IgE concentrations in BAL ranged from 12 to

151 ng/ml and averaged approximately 15% of the serum concentrations of total IgE at screening visit 1 (Table 6). Total IgE concentrations in BAL on Day 42 ranged from 45 to 169 ng/ml and averaged 21% of serum total IgE concentrations. Calculated ratios of E25:IgE from Day 42 BAL samples ranged from 18.3 to 2,000:1 (Table 6).

TABLE 6

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LUNG-DEPOSITED DOSES OF E25, BAL IgE LEVELS, AND E25:IgE RATIOS IN THE SUBGROUP OF 10 SUBJECTS WHO UNDERWENT BAL

The main finding of our study is that E25, a nonanaphylactogenic anti-IgE antibody, delivered by the aerosol route was no better than aerosolized placebo in attenuating the airway responses to inhaled allergen in allergic asthmatic subjects. This result is in contrast to previous findings in protocols where E25 was delivered intravenously.

The early phase response to allergen at Weeks 2 and 8 after initiation of treatment with aerosolized E25-1 mg daily was significantly less than the baseline early phase response, but the degree of attenuation was not significantly greater than placebo. The effects of E25-10 mg on the early phase response were no greater than the effects of E25-1 mg and also no greater than placebo. Similarly, analysis of data for the late phase response in the subgroup of subjects who had a significant late phase response at baseline showed no significant attenuation for either the 1 mg or the 10 mg dose groups. These data for the late phase response have to be interpreted very cautiously because of the small number of subjects with a late phrase response at baseline. Previously, in similar protocols using similar methods, we have shown that E25 administered intravenously significantly reduces both the early and late phase responses to inhaled allergen (1, 2). Thus, the aerosol route of delivery for E25 is not as effective as the intravenous route in attenuating airway responses to inhaled allergen.

There are at least three reasons for the lack of efficacy of aerosolized E25 in this study. First, the aerosol route of delivery may not have delivered sufficient E25 to the lower airways. E25 clearly reached the lower airways after aerosolization because E25 was detected in BAL and blood, and any swallowed E25 would have been inactivated in the stomach. However, it is possible that the aerosol route of delivery did not result in high enough concentrations of E25 to neutralize IgE in the lung tissue compartments surrounding IgE effector cells. The vascular space, in particular, represents a large "sink" of IgE constantly available to move into the lung interstitium to replace IgE complexed with E25. A second possible explanation for the lack of efficacy of aerosolized E25 in this study is that neutralizing antibodies to E25 developed and prevented E25 from binding free IgE. Antihuman antibodies have never been detected after intravenous administration of humanized E25 that contains less than 5% murine amino acid. However, the aerosol route of administration of E25 may be more immunogenic than the intravenous or subcutaneous route. A third possible explanation for the lack of efficacy of aerosolized E25 in this study is that the subjects were noncompliant. Compliance appears to have been good in this study, however, as evidenced by the counts of used and unused medication vials at each study visit.

In summary, we found that aerosolized E25 did not significantly attenuate the airway responses to

inhaled allergen in asthmatic subjects. One subject developed an IgG and IgA antibody against E25, suggesting that the aerosol route of delivery for monoclonal antibodies may be more immunogenic than the intravenous or subcutaneous route. We conclude that the aerosol route of delivery for E25 is not likely to be a useful treatment for allergic asthma.

Footnotes

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TOP
ABSTRACT
ARTICLE
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